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THE EFFECT OF ACUTE CHANGES IN BLOOD FLOW ON LOCAL LYMPH FLOW IN THE LIMBS OF ANESTHETIZED SHEEP

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ABSTRACT

A study was made of the effects of acute changes in local blood flow on lymph flow in the feet of anesthetized sheep. Lymph outflow pressure and flow and venous pressure were measured in cannulated vessels draining the foot region. Local volume changes were also measured. Acute reductions in blood flow were produced by sudden occlusion of the circulation to the limbs with a pneumatic cuff for periods of up to 1.5 hours. During the first 10 min of occlusion, there was little change in lymph flow; it then fell slowly and gradually, reaching about 25% of its control value after 40 min of arrest. On release of the circulation, lymph flow rose almost immediately to levels above the control value, resulting in a hyperlymphia whose size and duration was related to the duration of the circulatory arrest and the limb volume changes that followed. The results indicated that lymph flow can continue, albeit at reduced rates for long periods after circulatory arrest and that during reactive hyperaemia, there is a brisk hyperlymphia whose size and time course is similar to that of the limb volume changes.

There is little information on the effects of acute changes in blood flow on local lymph flow. Lewis and Winsey (1) studied the lymph flow responses to pharmacologically induced increases in blood flow in the cat hind limb. They found that some potent vasodilator drugs such as isoprenaline and prostaglandins had little effect on lymph flow. With others such as histamine, bradykinin and acetylcholine, intraarterial infusions did result in increases in lymph flow which started 5-7 min after the blood flow increased and peaked some 10-15 min later. When the drug infusions stopped, blood flow returned to normal within a few minutes but lymph flow might remain elevated after the infusion for hours. The results suggested that there was not very rapid coupling between the blood flow and lymph flow events and in some cases none at all.

The present study has looked at the relationship between blood flow and lymph flow in sheep limbs where spontaneous lymph flow can be continuously measured and local blood flow can be altered easily without drugs. Acute reductions in blood flow were produced by occluding the circulation with a pneumatic cuff around the leg and acute increases in blood flow were produced by the reactive hyperemias that followed. A short account of some preliminary experiments had been published (2).

MATERIALS AND METHODS

Experiments were carried out on 6 sheep anesthetized by induction with pentobarbitone (20-30mg/kg IV) and maintained on halothane (1-3% in 0_2). Lymphatics in the upper metacarpal region were cannulated against the direc-

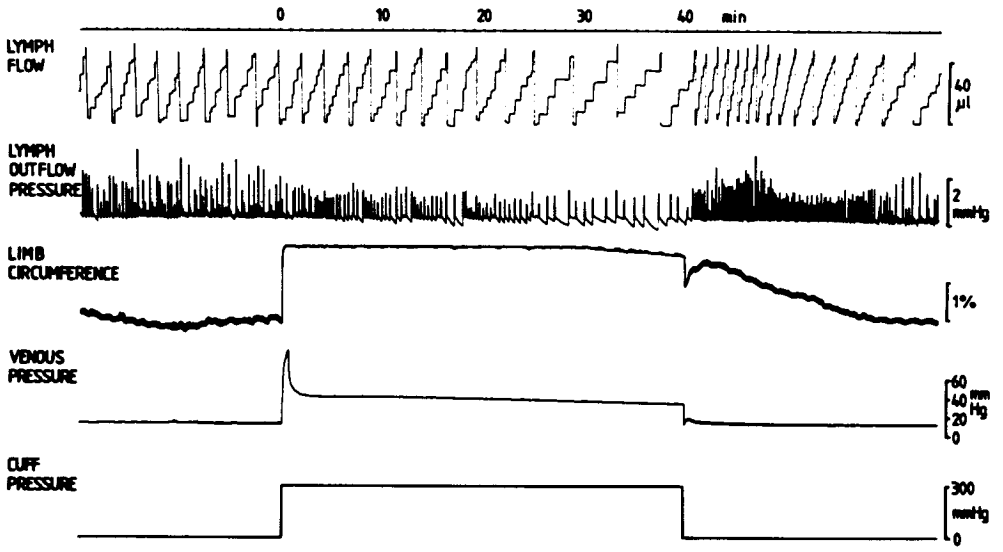

Fig. 1. Lymph flow, lymph outflow pressure, limb circumference, venous pressure, and occluding cuff pressure in the sheep's limb before, during and after occlusion of the limb circulation with a pneumatic cuff.

tion of flow with a 5-10cm piece of PVC tubing (0.28-0.5mm ID). To measure lymph flow, this was connected by a 30-50cm length of larger bore (0.9mm ID) tubing to the side arm of a tension transducer at the same level as the cannulated lymphatic. A T piece in the larger tubing led to a pressure transducer to measure lymph outflow pressure as described by McHale and Roddie (3). Limb volume was estimated from measurements of limb circumference using a strain gauge plethysmograph (Medasonics, model SPG16) applied between the hoof and the fetlock joint. Venous pressure was measured in some experiments via a catheter inserted into a metacarpal vein. The circulation to the limb was occluded by a pneumatic cuff inflated to 200mmHg above the knee joint. The site was above the site of the lymphatic cannulation so that it did not interfere with lymph drainage from the cannulated lymphatic.

RESULTS

Fig. 1 shows the record of an experiment in which the circulation was arrested for 40 min. The records from above downwards are lymph flow, lymph outflow pressure, limb circumference, venous pressure and occluding cuff pressure. At the beginning of the experiment, the lymphatic contracted at about 2-3 beats/min and with each beat a small amount of lymph was expressed which appeared as a step increment on the flow record. This accumulated as a drop on the sidearm of the tension transducer to produce a cumulative flow record. When the drop reached a certain size, it dropped off and the tension transducer reset. The steepness of the ramps was a measure of the lymph flow rate, in this case about 22µl/min.

Circulatory arrest did not cause much change in lymph flow during the first 10 min of occlusion. However, after about 10 min, the rate of beating and the level of lymph flow began to fall steadily. After 40 min of occlusion, the contraction rate had fallen to about 9 beats/min, less than half the control value. After long periods of circulatory arrest, it was noticed that not only did the rate of spontaneous contraction in the lymphatics get slower but there was also a decrease in the rate of rise and in the rate of fall in

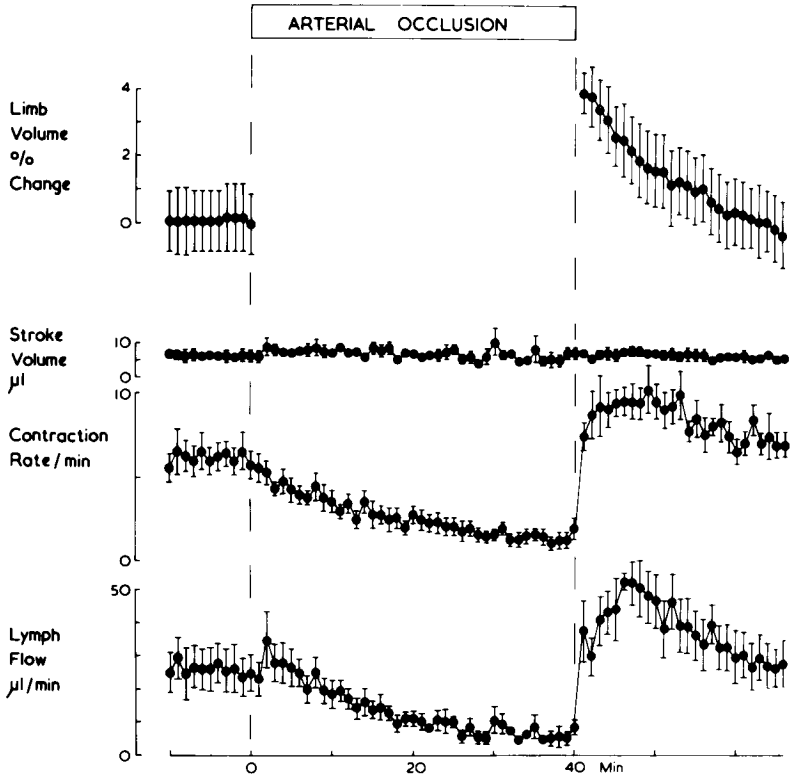

Fig. 2. The effect of 40 min arterial occlusion on limb volume, lymphatic stroke volume, lymphatic contraction rate, and lymph flow in the sheep's limb. Each point is the mean of six observations on each of six sheep; the vertical bars showing ± 1 SEM.

tension during the contractions. When the circulation was occluded, venous pressure and limb volume rose immediately and stayed elevated during the period of circulatory arrest. Release of circulatory arrest resulted in an increase in the rate and amplitude of the lymphatic contractions after a latency of about 30 sec and this was associated with an increase in lymph flow to more than double the control level. The hyperlymphia peaked about 3 min after release of the circulation and then flow gradually returned towards the resting level over a 20 min period. The time course of the lymph flow changes was similar to that of limb circumference as recorded by the strain gauge.

Fig. 2 shows the averaged results of six experiments of this sort on six sheep. Before occlusion, the mean flow rate was about 25µl/min, the mean lymphatic contraction rate about 6 beats/min, and the mean stroke volume about 5µl. When the circulation was arrested, lymph flow was little affected for the first 5-7 min of the occlusion. It then began to fall slowly and continued to do so during the remainder of the period of circulatory arrest. It had fallen to mean values of about 10µl/min after 20 min and to about 5µl/min after 40 min. The fall in flow was associated with a fall in the contraction rate from about 5 to about 1 beat/min; there was little change in stroke volume. When the occlusion cuff was deflated, lymph flow increased rapidly from a mean of about 5 to about 35µl/min in the first minute. It continued to rise for the next 5 min and then returned gradually to normal over the next 20 min or so. The lymphatic contraction rate also rose sud-

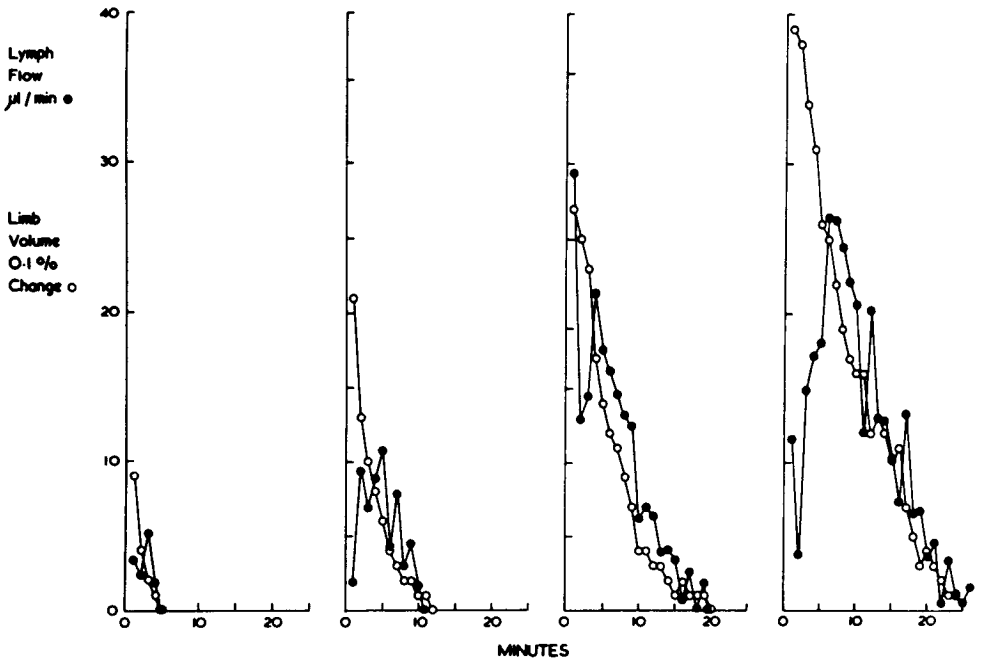

Fig. 3. A comparison of the increases in lymph flow and in local limb volume in the sheep's foot following (from left to right) 5, 10, 20, and 40 min of arterial occlusion. Each point is the mean of six observations on each of six sheep.

denly on release of occlusion. After release of the occlusion cuff, the volume of the limb was greater than that in the preocclusion control period, and returned gradually to the control level with a time course similar to that of the lymph flow.

Fig. 3 shows a comparison of the mean sizes and durations of the increases in lymph flow and in limb volume above their control levels following circulatory arrest for 5, 10, 20, and 40 min. Each point is the average value obtained in the six experiments on the six sheep. As the duration of the circulatory arrest increased, so did the size and duration of the subsequent hyperlymphia and increase in limb volume. After 5 min occlusion, volume increased by about 1%, lymph flow by about 5µl and both took about 5 min to return to the control value. After 40 min occlusion, volume increased on average by about 4%, lymph flow by about 25µl/min, and both took about 25 min to return to normal. Whereas the volume changes peaked in the first minute after release, in most cases the lymph flow peaked some 4-8 min later.

DISCUSSION

Though the experiments are not quite comparable, these results are in keeping with those of McMaster (4) who looked at the effect of venous occlusion on lymph flow in human forearm skin using the formation of streamers of dye injected subcutaneously as an indication of lymph flow. When the veins were occluded by inflating a cuff to 90mmHg around the upper arm for 25 min, there was little evidence of streamer formation suggesting that lymph flow was low. However, when the cuff was released, streamer formation became marked during the period of reactive hyperemia that followed indicating an increase in lymph

flow and formation. It is likely that the raised venous pressure increased tissue fluid formation and so encouraged lymph formation, but if it did, it did not increase lymph flow during the actual period of congestion. He concluded that rapid lymph movement takes place during the hyperemia that follows a period of venous obstruction.

Lymph flow during circulatory arrest

In these experiments lymph continued to flow, albeit at a reduced rate, for a long time after the circulation had been arrested. In some experiments it was still flowing 1.5 hrs later. The length of time the lymphatics continued to pump after circulatory arrest seemed to depend on the initial level of flow. When the initial level of lymph flow was low, e.g., 1μl/min, lymph flow ceased about 10 min after the start of circulatory arrest (2). For the first 5-10 min after circulatory arrest, the lymph flow did not fall; in most cases, it was slightly raised though this rise was not significant. The reason for this is unclear. However, there was a transient peak followed by a sustained rise in venous pressure following application of the occlusion cuff and rises in venous pressure have been shown to increase lymph flow in sheep limbs (5). Venous pressure rises when the circulation is suddenly occluded; when flow across the post-capillary resistance vessels is suddenly stopped, there is no pressure drop across these vessels and venous pressure rises towards capillary pressure (6).

The lymph being evacuated during circulatory arrest was presumably derived from the interstitial fluid reservoir which is normally replenished by the circulation; depletion of the interstitial reservoir is a possible explanation for the fall in lymph flow after circulatory arrest and why it may fall to zero if the initial rate of lymph flow is low. The fall in contraction rate could have been due to a decrease in the amount of lymph being presented for pumping, but a fall in limb temperature and local tissue oxygen tension in the occluded limb may have contributed to the fall. These factors may also have contributed to the slowing of the rate of rise and the rate of fall of tension in the lymphatic contractions during prolonged circulatory arrest.

It has been suggested that blood vessel pulsation is important for the formation of lymph and for the maintenance of lymph flow (7). However, in the present experiments, lymph continued to flow in quiescent limbs of anesthetized sheep in which all transmitted pulsations were abolished by the occluding cuff. This is in keeping with the finding that lymph formation in sheep limbs is not reduced when the perfusion pressure is changed from a pulsatile to a steady one with the same mean value (8).

The fact that fluid can move from the interstitial space to the lumen of the initial lymphatics in the absence of movement, pulsation or pressure gradients which depend on a local circulation must be taken into account when considering the mechanism of lymph formation. If the forces needed to form lymph are not generated by the movements of surrounding structures or by pressure gradients generated by the circulatory system, it may be that the initial lymphatics themselves are capable of generating transmural pressures by their intrinsic activity (9). It is clear that tissue movement (10) and large transmural pressure gradients across the wall (11) of initial lymphatics will increase lymph formation in the sheep's foot but the present experiments suggest that a mechanism is also needed to explain lymph formation in the absence of such force.

Lymph flow during reactive hyperemia

When the circulation to a limb is restored after a period of circulatory arrest, there is a "reactive hyperemia" (12) which consists of a rapid rise in blood flow to above the resting level followed by a gradual fall back to that level. This is thought to be due to the vasodilatation of the resistance vessels caused by metabolites which accumulate in the tissue dur-

ing the period of circulatory arrest. The size and duration of the hyperemia is roughly proportional to the duration for the occlusion (13) though the relationship between the blood flow debt during arrest and the blood flow repayment during reactive hyperemia is not a precise one (14).

In these experiments, there was a surprising contrast between the slowness with which lymph flow declined when the circulation was arrested and the briskness with which it rose again when the circulation was reestablished. There was a dramatic increase in lymph flow after a period of circulatory arrest and the size of this hyperlymphia was related to the duration of the circulatory arrest as is the size of the reactive hyperemia (13). It is important to remember that the increased lymph flow after circulatory arrest was not due to the sudden release of lymph dammed up in the lymphatics by the arterial occlusion cuff. The lymphatics were cannulated *distal* to the occlusion cuff and were not obstructed by it; lymph continued to flow from the lymphatics during the period of circulatory arrest.

We do not know the reason for the briskness of the hyperlymphia during reactive hyperemia. Pressure in the afferent lymphatic rose markedly sometimes with 10 sec of the pneumatic cuff being deflated. It is not likely that once the circulation is restored, fluid can pass out of the capillaries, through the interstitium, into the initial lymphatics and up the afferent lymphatics in as little as 10 seconds. Nevertheless, there was close coupling between the lymph flow change and the volume change during reactive hyperemia which is an index of the change in local blood flow. Another possibility is that the fall in limb temperature and tissue oxygen tension in the ischemic limb so interfered with the contractile capabilities of the lymphatic smooth muscle that the lymphatics were unable to clear the lymph presented to them during the period of circulatory arrest so that this lymph accumulated in the vessels. It was noticed that the rate of rise and fall of tension in the lymphatic contractions slowed markedly as the circulatory arrest continued. It is possible that the sudden increase in local temperature and tissue oxygen tension due to the inrush of blood during reactive hyperemia rapidly restored the contractility of the lymphatic smooth muscle so that the lymphatics could propel the lymph they had failed to clear during the period of circulatory arrest and that this accounted for the brisk hyperlymphia.

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